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Brief Communication: Association Between Cytosolic Low Molecular Weight Phosphotyrosine-Phosphatase and Malaria—A Possible Mechanism

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ABSTRACT Cytosolic low molecular weight phosphotyrosine-phosphatase shows dephosphorylating activity of the band 3 protein. Increased phosphorylation of this protein increases membrane rigidity and resistance to invasion of red blood cells by malarial parasites. This observation may explain the negative association previously reported by our group between the high activity *C allele of cytosolic low molecular weight phosphotyrosine-phosphatase and past malarial morbidity. Am J Phys Anthropol 108:241–244, 1999. © 1999 Wiley-Liss, Inc.

An association between past malarial morbidity and cytosolic low molecular weight phosphotyrosine-phosphatase (cLMW-PTP/ACP1) genotype was reported by our group more than 20 years ago (Palmarino et al., 1975; Lucarelli et al., 1976). Both in Sardinia and the Po Delta, a lower frequency of ACP1*C was observed in populations in which a high malarial prevalence had been endemic compared to nearby populations that had always been free of malaria. Moreover, in highly malarial endemic areas of the tropics, very low *C frequencies had been reported. The *C allele is associated with the highest ACP1 activity and with the highest concentration of the slow fraction (s isoform). At that time, it was not possible to propose an explanatory mechanism for these observations. Since then, important functions of ACP1 have been elucidated, and some mechanisms of resistance to malaria have been described suggesting a possible explanation for such association.

MATERIALS AND METHODS

Data on 1,640 children aged 7–14 living in 14 Sardinian villages located at various altitudes in the central area of the Island have been reconsidered (Palmarino et al., 1975).

The ACP1 genotype had been determined according to Hopkinson et al. (1963). The f and s isoforms and the total enzymatic concentration (micrograms per milliliter of packed red blood cells [RBC]) were assigned to ACP1 genotypes according to the Dissing method (Dissing, 1993; Bottini et al., 1995).

The distribution of malaria in individual Sardinian villages was investigated by Fermi prior to 1940 (Fermi 1934, 1938, 1940), and in 1947–1948 a survey was carried out in 66 villages by the organization ERLAAS, which was in charge of an eradication campaign (Logan, 1953).

Malarial morbidity is reported as percent parasitic rate. Fermi's data include all *Plasmodium* species. The ERLAAS observations, however, indicate that the prevalence of *Plasmodium falciparum* paralleled that of all *Plasmodium* species.

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TABLE 1. Percent distribution of ACP1 alleles in Sardinian villages grouped according to intensity of past malarial morbidity

	Past malarial morbidity (parasite rate %)		
	>80	≤80 > 20	≤20
ACP1*A	21.28	25.39	17.70
ACP1*B	71.96	65.52	69.42
ACP1*C	6.76	9.09	12.88
Total number of alleles	1,198	638	1,390
Number of villages	4	4	6
Chi-square test of independence	$P < 0.0001$		

Correlation analysis between ACP1 isoforms and past malarial morbidity was performed both on single villages and on grouped villages. The choice of cut-off points was based on following criteria: 1) to have comparable numbers of villages in each group and 2) to evaluate ACP1 parameters at extreme values of past malarial morbidity.

RESULTS

Table 1 shows the distribution of ACP1 alleles in Sardinian villages grouped according to past malarial morbidity. Figure 1 shows the relation between ACP1 isoforms and past malarial morbidity for the grouped villages. For single villages, a correlation coefficient of -0.71 was obtained between past malarial morbidity and the s isoform ($P < 0.01$).

The most relevant information obtained from our revisitation of the Sardinian data can be summarised as follows. 1) In Sardinia there is a significant negative correlation between ACP1 s isoform concentration and past malarial morbidity. The populations subjected in the past to a heavy malarial burden now show a lower concentration of the s isoform compared to nearby malaria-free populations. This suggests that genotypes with a high s isoform concentration have been subjected to negative selection in a malarial environment. 2) Correlation analysis and analysis of the joint Gd-ACP1 distribution suggest that the relationship between endemic malaria in the past and the s isoform has not been mediated by G-6-PD deficiency, which indicates a direct effect of malaria on ACP1 (data not shown).

DISCUSSION

Band 3 protein (B3P) phosphorylation and molecular organization of the erythrocyte membrane may have an important role in the resistance to malaria

Erythocyte ovalocytosis is associated with reduced susceptibility to malaria. The cytoplasmic domain of the ovalocyte band 3 protein (B3P) is approximately 3 kDa larger than the normocyte protein and shows a markedly increased phosphorylation. Structural alteration of B3P is the cause of increased phosphorylation, increased membrane rigidity, and resistance to invasion by malarial parasites (Jones et al., 1990). It has been also observed that a monoclonal antibody directed against the cytoplasmic portion of B3P can induce an increase in the immobile fraction of B3P and will inhibit the invasion of erythrocytes by malarial parasites (Tilley et al., 1990, 1991).

ACP1 is a phosphotyrosine-phosphatase (PTPase) having two isoforms f and s that show different cynetic parameters for their dephosphorylating activity of B3P

Boivin and Galand (1986) were the first to show that ACP1 dephosphorylates a tyrosine residue of the erythrocyte membrane protein B3P. Recent experiments have shown the following cynetic parameters of f and s isoforms for phosphorylated B3P: $K_m = 1.4$ for F and 0.4 for S; $V_{max} = 68.0$ for f and 10.5 for s (Stefani et al., 1993).

A possible mechanism for association between ACP1 and malaria

High PTPase activity may lower the degree of B3P phosphorylation, thus decreasing membrane rigidity and favouring the invasion of the cell by the parasite.

This mechanism is suggested by the association between B3P phosphorylation and resistance to invasion by malarial parasites that is observed in ovalocytosis. In this case, however, the increased phosphorylation does not depend on decreased PTPase activity but on the structure of the B3P variant. On the other hand, since f and s isoforms have different affinities for this substrate, it is conceivable that a high concentration of the s isoform might induce conformational

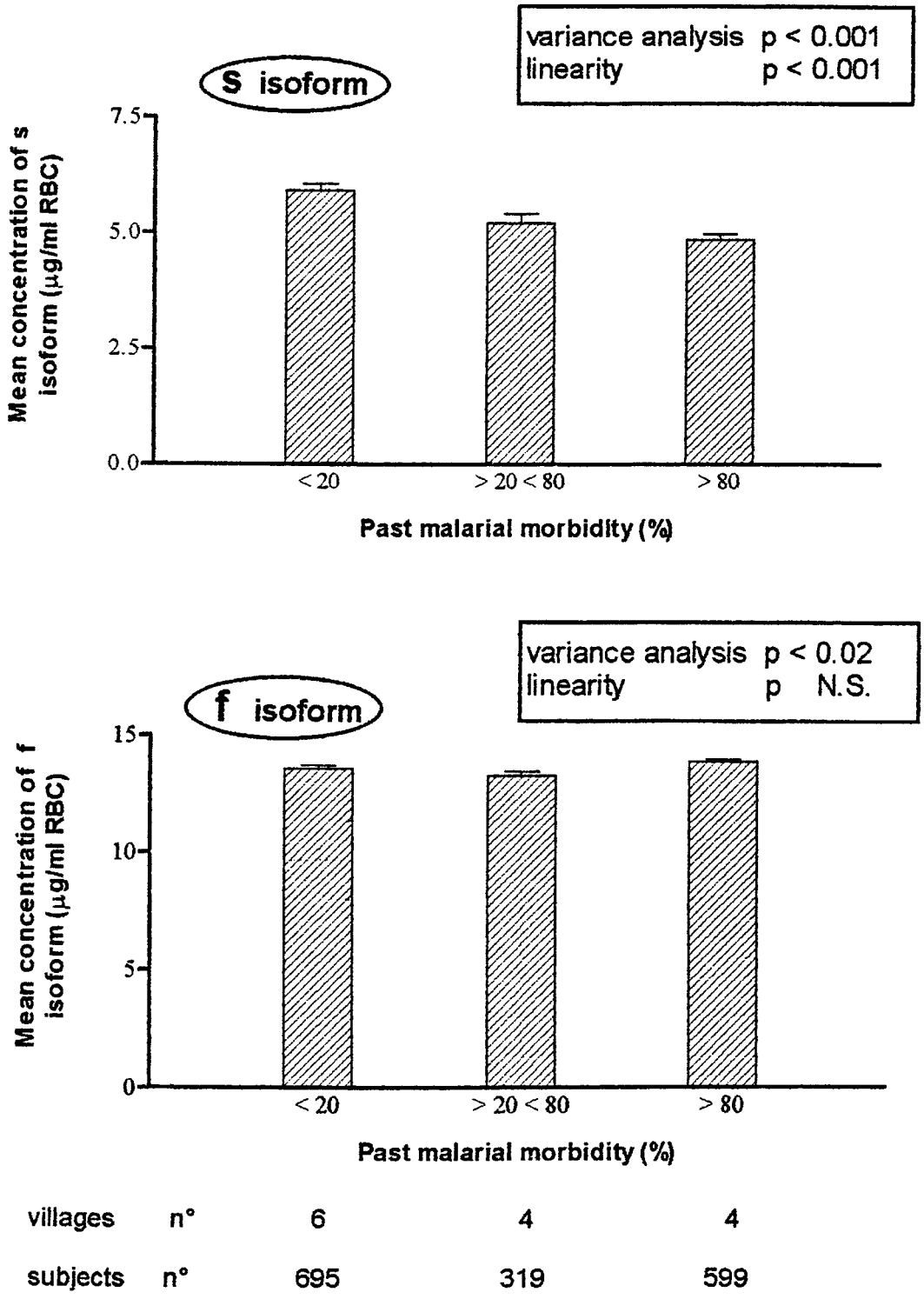

Fig. 1. The relationship between past malarial morbidity and ACP1 isoforms in Sardinia.

changes of B3P that decrease erythrocyte resistance to *Plasmodium*.

The hypothesis can be tested by the study of *Plasmodium* development in red blood cells with different concentrations of the s isoform. f and s isoform activities are differentially modulated by several natural and synthetic chemicals (Wurzinger et al., 1985; Dissing, 1993); such substances might prove useful in modifying the susceptibility and the clinical course of malaria.

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